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SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA

DYSENTERY IN HAZARIBAGH CENTRAL JAIL JANUARY 1910—MARCH 1911

BEING THE REPORT OF AN ENQUIRY

CARRIED OUT BY

CAPTAIN R. T. WELLS, M.A., M.B., I.M.S.

Under the direction of the Director, Central Research Institute, Kasauli

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE
SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA



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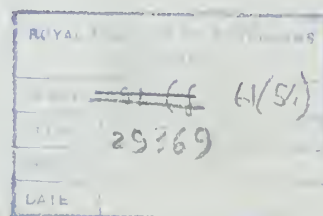
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NOTE.

This enquiry was undertaken at the instance of the Inspector-General of Prisons, Bengal, with the sanction of the Local Government. The Superintendent, Central Jail, Hazaribagh, very kindly made arrangements for a temporary laboratory in the jail and gave every facility for the work.

AN ENQUIRY ON DYSENTERY IN HAZARIBAGH CENTRAL JAIL,

JANUARY 1910—MARCH 1911.

I. Introduction.

(a) Dysentery in general.

The term "dysentery" cannot be taken to indicate a single specific disease or even a group of specific diseases; it simply connotes a rather ill-defined collection of symptoms and is generally applied to cases which, at some period of their course, present blood and mucus or mucus alone in the stools, with or without griping and tenesmus and with or without initial rise of temperature.

The local symptoms point to an inflammation of the intestine, principally or entirely confined to the large gut, while the general disturbance of health is due to defective nutrition and to the absorption of toxins from the intestine.

Amidst the confusion which at present obtains in our knowledge of the dysenteries, two types, "bacillary" and "amœbic" are generally recognised as specific; other cases are considered as part of a general infection such as Malaria or Kala azar. Again, in many cases a pathogenic role has been assigned to a variety of other organisms, both bacteria and protozoa, while in a large proportion of cases the specific factor in etiology has so far eluded observation.

The dysenteries may be provisionally classified as follows :—

- (i) Specific { (a) Bacillary.
 (b) Amœbic.
- (ii) Non-specific { (a) Tubercular.
 (b) Malarial.
 (c) Due to Leishmania infection.
 (d) Verminous (Manson).¹⁴
- (iii) Dysentery of uncertain origin.

I. BACILLARY DYSENTERY.

Bacillary dysentery is described as an acute specific disease, generally occurring in well-defined epidemics, due to an infection with the bacillus dysenteriae (Shiga) or allied organism.

The natural incubation period is 2-3 days; in accidental laboratory infection in man, 24-48 hours. The disease generally lasts for 4-8 days in slight cases, 3-6 weeks in severe cases.

Reinfection is rare when the course of the disease has been severe, a pronounced attack apparently conferring immunity.²⁰

Water, milk, food and flies have been brought forward as possible agents in the spread of the disease, but "bacillus carriers" are generally considered as the principal source of infection.²¹

The large intestine is generally alone infected but the lower ileum may also share in the disease. The inflammation is diphtheritic in character, affecting first and foremost the colic flexures and the rectum on the summits of the folds of the mucous membrane.

Actual ulcers are rare, but when they do occur, each has a floor co-extensive with its surface, the edges not being undermined.

The liver generally shows no marked change.

Although bacillary dysentery as it occurs in Europe is described as an acute epidemic disease, Böse³ notes that in Manchuria it often runs a chronic course and is difficult to distinguish clinically from amœbic dysentery.

Forster⁹ also found chronic bacillary cases in Midnapore Jail, Bengal.

2. AMŒBIC DYSENTERY.

Amœbic dysentery is described as a chronic endemic disease of insidious onset due to infection of the large intestine with pathogenic amœbæ.

The course of the disease is very irregular, convalescence being often interrupted by relapses. One attack does not confer immunity; on the contrary it appears to predispose to subsequent attacks.²²

The disease is generally considered to be water-borne (Strong)²² but Viereck²⁴ considers contact infection as the principal means of spread.

While the large intestine is the main site of the disease, the lower ileum may also, in rare cases, be affected, as in bacillary dysentery.

The changes in the intestinal wall, in the earlier stages at least, are in striking contrast to those described under bacillary dysentery.

The initial lesions consists of a hæmorrhagic catarrh, with raised nodules protruding above the level of the surrounding mucosa. These nodules may be as small as a hemp-seed or as large as a bean; the smaller ones are hemispherical, the larger oval in shape and they are most frequently situated on the summits of the mucosa.

Such a nodule may break down, one or more perforations occur at the apex, forming a narrow neck of communication between the cavity of the nodule and the lumen of the gut. Thus the ulcer "en bouton de chemise," a characteristic lesion of amœbic dysentery, is formed. The amœbæ are found in the ulcers and in the surrounding tissues (Dopter).⁸

A superposed infection of non-specific bacteria may give rise to a diphtheritic inflammation such as occurs in specific bacillary dysentery.

Liver abscess is a frequent complication (11-33 per cent., Strong),²² the amœbæ being demonstrable in the contents and walls.

With regard to the details of the morphology and life-cycle of pathogenic amœbæ, much confusion and radical difference of opinion among observers at present exists.

A form of dysentery associated with the presence of motile amœbæ in the stools had long been recognised, but, in 1903, the work of Schaudinn¹⁹ first gave definition to the subject and indicated lines of research which have since been followed by several other observers.

He distinguished two types of intestinal amœbæ (i) *Entamoeba coli*, a harmless commensal, and (ii) *Entamoeba histolytica*, identified by him with that previously described and figured by Jürgens¹² as the causal organism in three cases of dysentery originating in China.

He did not find that these amœbæ could be cultivated outside of the body and based his description of them on the morphology and life-cycle as studied by him in the intestinal contents and, in the case of *E. histolytica*, in the intestinal wall of men who had died of dysentery and of experimentally infected cats.

Viereck²⁴ in 1907, Hartmann¹⁰ in 1908 and Werner²⁷ also in 1908, following Schaudinn's methods of observation, described another species of amœbæ in the stools from cases of dysentery, (*Entamoeba tetragena*) allied to *E. histolytica*.

Viereck²⁴ considered that the natural occurrence of pathogenic amœbæ in water had not been convincingly demonstrated, and that such amœbæ had not been proved to multiply on artificial media; on the contrary, he emphasised the fact that the motile amœbæ found in stools appear to die off rapidly after their discharge from the body.

Werner²⁷ attempted to secure multiplication of parasitic amœbæ outside the body by transferring to fucus agar medium fresh stools containing vegetative forms of *E. histolytica* and *E. tetragena*: in no case did he see multiplication of these amœbæ; (the number of observations is not stated). On the other hand, he found on the agar medium, growth and encystment of what was apparently a different species of amœba; this organism, which was characterised by the presence of a contractile vacuole in the vegetative phase and by round double-contoured cysts in the resting condition, he was inclined to identify with *Amœba limax*, (Vahlkampff), a free living form.

He shewed that when cysts of this amœbæ were artificially fed to house-flies, such cysts passed through the alimentary canal apparently unchanged so

that they germinated when the fæces of the infected fly were transferred to a suitable medium.

He suggested that the amœbæ appearing in cultures from human fæces might have had a similar history.

In marked opposition to the conclusions of the above observers are these of Musgrave and Clegg, Lesage, Walker and Noc.

In 1904 Musgrave¹³ and in 1906 Musgrave and Clegg,¹⁶ using an agar medium with an alkalinity of 1 per cent., cultivated amœbæ from the stools in cases of dysentery in Manilla, from water, soil and a variety of outside sources.

They did not discover, in any these amœbæ from different sources, characters which would serve to distinguish different species amongst them or finally to distinguish these cultivated forms from those found microscopically in the infected intestine and liver.

They considered it probable that any free-living amœba might, under certain conditions, become a pathogenic parasite.

In 1905, Lesage¹³ stated that he was able to obtain, in 7 out of 20 cases of tropical dysentery, cultures of amœbæ from the stools, using washed gelatine as a medium. These amœbæ he considered as identical with *E. histolytica* (Schaudinn).

In 1908, Walker²⁵, using Musgrave's medium, obtained from fæces of different animals 44 cultures of what he considered parasitic amœbæ, among which he distinguished 10 species; he did not succeed in obtaining a culture from human fæces.

Noc,¹⁹ in 1909, by the use of a gelatine medium 0.5 per cent. alkaline in reaction obtained cultures of amœbæ from (i) liver abscess pus, (ii) the stools in cases of dysentery, (iii) the water supply of Saigon (French Indo-China); these amœbæ he considered to belong to one single species, identical with that found microscopically in the fæces and in liver abscess pus, and he looked upon them as the causal organism of amœbic dysentery and hepatitis.

In the medical literature on amœbæ, as will be seen from the above resumé, opinion is sharply divided on one main issue, as to whether the amœbæ visible microscopically in the stools are or are not specifically identical with those which can easily be cultivated on artificial media.

For the affirmative, Musgrave and Clegg, Lesage, Walker and Noc; for the negative Schaudinn, Jürgens, Viereck, Hartmann and Werner are quoted above.

The following are contributions to the question from the point of view of the protozoologist: Vahlkampf²³ mentions that Kartulis cultivated a "dysentery" amœba in a medium consisting of diluted rabbit's and pigeon's fæces contained in open vessels, and that Kruse and Pasquale considered that

these amœbæ were not parasitic but were simply saprophytes which had gained access to the cultures from the air.

Nägler,¹⁷ (1909), in introducing observations on the life-cycle of some saprophytic amœbæ, says "In medicine also, an exact knowledge of the life-cycle of amœbæ is of great importance; it precludes such error as has arisen in the work of Musgrave and Clegg, for example; these authors have cultivated forms resembling *Amœba limax* and have given them out as dysentery amœbæ, whereas these cultivated forms have absolutely nothing to do with genuine parasitic amœbæ, but belong to the *limax* group."

Doflein,⁷ (1909), after considering the various amœboid organisms which have been described as occurring in the fæces, dwells upon the difficulty of coming to a decision as to which of the following headings such organisms should come under—

- (i) "Gelegentliche Passanten des Darms" *i.e.*, casual visitors in the gut or pseudoparasites: this group would include saprophytic amœbæ swallowed in the encysted condition and excreted unchanged²⁷
- (ii) Facultative parasites, and (iii), amœbæ specifically adapted to the parasitic habit.

Recently (March 1911), Whitmore²⁸ examined cultures on Musgrave's medium of amœbæ from fæces and liver abscess pus obtained in Manilla. In a preliminary note he states that he found these amœbæ to represent two distinct types, both of which belonged to the "*limax*" group; the vegetative forms possessed "the typical karyosome nucleus" and formed cysts with one single nucleus. He concludes by confirming Hartmann's view that they have nothing whatever to do with the parasitic amœbæ which occur in the gut.

Major W. G. Liston, I.M.S., in a paper as yet unpublished which he has kindly allowed me to read in manuscript, describes two different kinds of amœbæ which he had separated in a growth from liver abscess pus on Musgrave's medium; he is of opinion that types similar to both of these forms are described by Noc as belonging to a single species.

3. NON-SPECIFIC DYSENTERIES.

Infection of the large intestine by the tubercle bacillus may give rise to symptoms hard to distinguish clinically from chronic dysentery (Buchanan).⁴

Malarial and *Leishmania* infections are also stated to give rise occasionally to symptoms of dysentery (Manson).¹⁴

Another form of the disease due to worm infections is also described by the same author as "Verminous dysentery."¹⁴

4. DYSENTERIES OF UNCERTAIN ORIGIN.

Apart from the members of the "B. dysenteriae" group, various bacteria have at times been described in causal relation to dysentery.

A virulent type of *B. coli* pathogenic to animals, was isolated from dysenteric stools by Chantemesse and Widal, Arnaud and others; streptococci and *B. pyocyaneus* have also been credited with causing dysentery.⁶

Possibly dysenterigenic properties have also been attributed to several flagellates, *Lamblia intestinalis*, (Bohne u. Prowazek),² *Balantidium* (Hartmann)¹¹ and *Trichomonas* (Billet).¹

It does not appear, however, that the claims of any of the above organisms to rank as dysentery-producers have been established.

It is, indeed, widely recognised that many cases of dysentery occur in which microscopical and cultural examination of the stools during life and of pathological material after death fail to demonstrate a causal organism.

Strong²² remarks that, (besides amœbic and bacillary forms), "there is a third catarrhal dysentery which is seen occasionally in the tropics and has a varied etiology. It is occasionally indistinguishable from amœbic dysentery."

Shiga²¹ notes that many cases of chronic dysentery come under observation in which neither dysentery bacilli nor amœbæ are to be found; these he characterises as "subsequent diseases," presuming that the causal organisms originally present have been eliminated from the gut, leaving unhealed lesions behind.

Davidson⁶ states that "symptoms clinically indistinguishable from specific bacterial dysentery also arise from chill, dietetic errors and mechanical, toxic or parasitic irritants," for such cases he suggests the title "pseudo-dysentery." Manson,¹⁴ (1907) remarks "we cannot even say for certain whether there is but one disease having grades of severity or a dozen specifically distinct diseases included under the term "dysentery."

5. MALINGERING.

Malingers must naturally be common in Indian Jails (Buchanan) and dysentery offers one of the best subjects for their art.

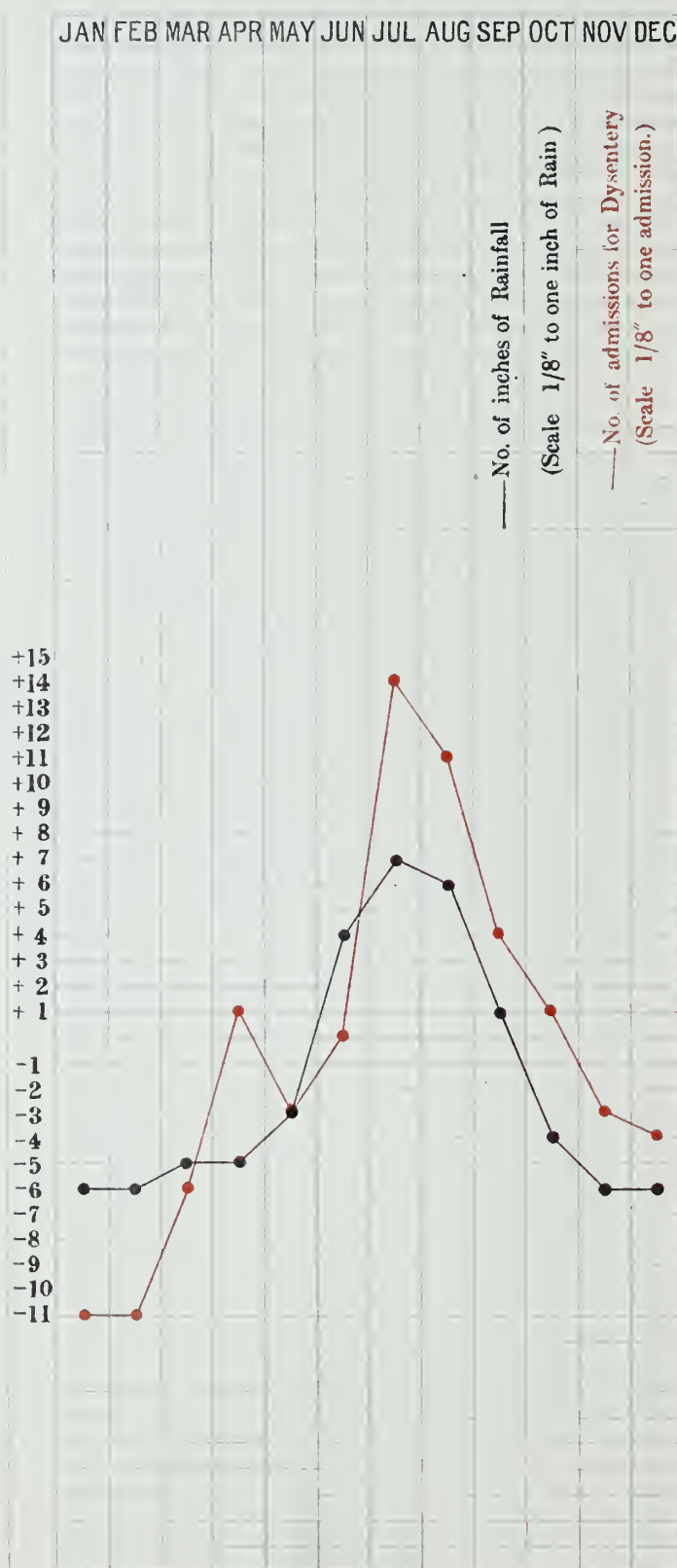
The principal irritants used by convicts to produce or aggravate dysentery seem to be:—

- (i) *Mechanical*, such as, powdered glass or brick dust, iron filings, chaff.
- (ii) *Chemical*, as aloe-juice, crushed castor or mustard seeds, crushed marigold heads, raw garlic, (the core of the latter being used as a suppository), coal-tar, "Saji Matti" (alkaline earth used in washing clothes) and lime.

**Charts representing the departure from the mean of the average
Monthly admission rates for Dysentery and Diarrhoea in
relation to the rainfall during the years 1904–1909.**

**I.
In 14 Bengal Jails.**

Chart I.



**Mean of average Monthly admission rates
for Dysentery and Diarrhoea 32.**

Mean of average monthly rainfall 6.

Moreover, the practice of substituting dysenteric stools for their own more or less healthy motions is not unknown among convicts and healthy stools may be mixed with urine and blood in order to simulate dysenteric motions.

(b) Intestinal Diseases in Bengal Jails.

In Bengal Jails intestinal disturbances account for a much larger proportion of admissions to hospital and of deaths than diseases of any other single system.

As already pointed out the term "dysentery" is a loose one ; in the early stages of the disease, as also in chronic cases, diarrhœa is often a marked feature and cases may be diagnosed according to the official "Nomenclature of Diseases" as "14. Epidemic diarrhœa" or "540. Diarrhœa" while "515 (1) Enteritis" and "515 (3) Colitis" in the returns may be taken as largely synonymous with "11. Dysentery."

In all Bengal Jails, from 1904-1909, there were 7,833 admissions for "Diarrhœa" with 99 deaths (1·35 per cent.), the case-mortality for "Dysentery" during the same period being 3·12 per cent. Intestinal diseases with even the former percentage of fatalities probably include many cases which might equally well have been returned as "Dysentery."

During the period 1904-1909 the annual average of the total number of admissions for dysentery and diarrhœa in Bengal Jails was 3,930·2 with 102·3 deaths, as compared with a total of 13,687·3 admissions and 333·8 deaths from all causes, so that 28·7 per cent. of all admissions and 34·2 per cent. of all deaths were due to dysentery and diarrhœa.

The disease incidence shows a marked seasonal variation, generally reaching a maximum in July (see Chart No. I).

As shown by Colonel W. J. Buchanan, I.M.S., there had been in 1909, a marked and progressive decrease in the admissions for dysentery (400 per mille to 145 per mille) as also in the case-death-rate (5 per cent. to 2 or 3 per cent.) in Bengal jails collectively during the previous 30 years ; but there still occur in individual jails fluctuations in the incidence of the disease, the causes of which at present remain obscure.

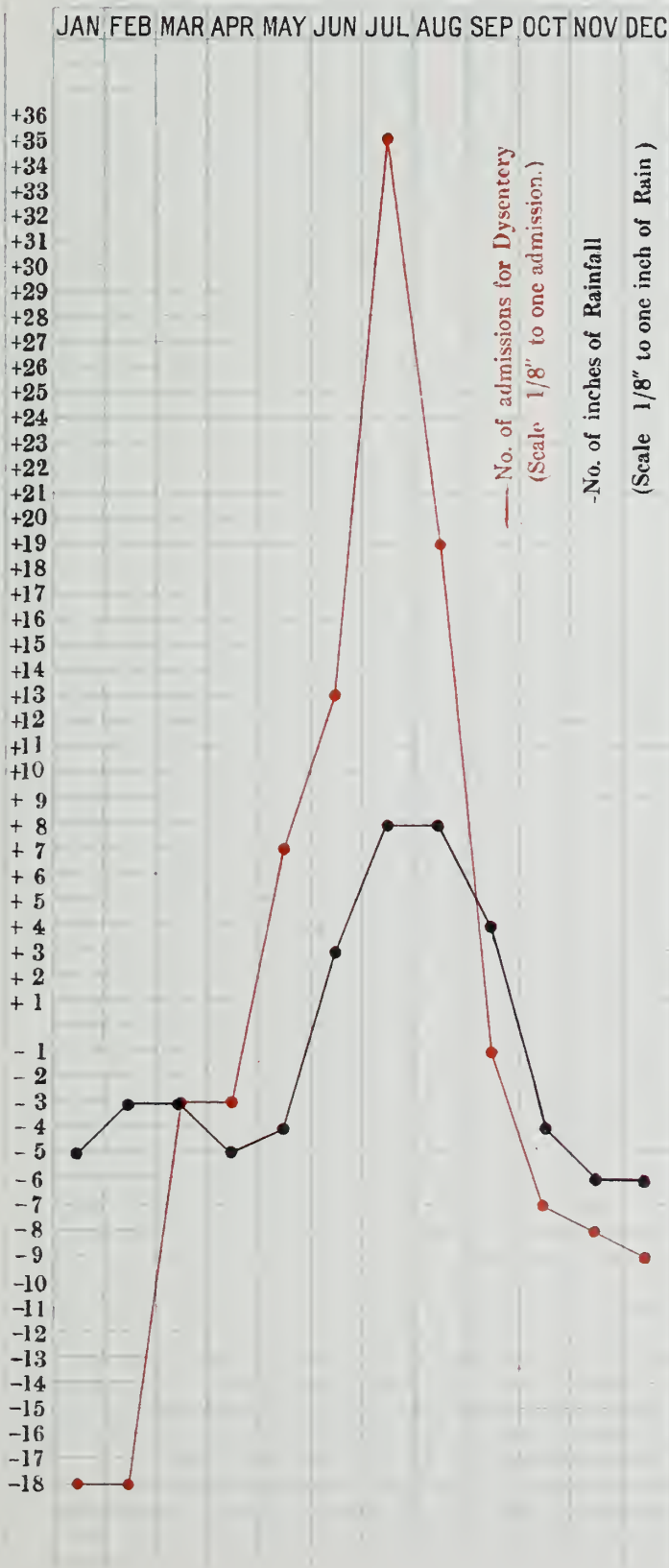
(c) Dysentery in Hazaribagh Central Jail.

Over the period 1904-1909 the average maximum prevalence of dysentery and diarrhoea in Hazaribagh Central Jail occurred, as in Bengal Jails generally, in July (Chart No. II) and in 1910 also the "paroxysmal" July rise in the curve is very striking (Chart No. III).

Charts representing the departure from the mean of the average
Monthly admission rates for Dysentery and Diarrhœa in
admission to the rainfall during the years 1904–1909

II.
In Hazaribagh Central Jail.

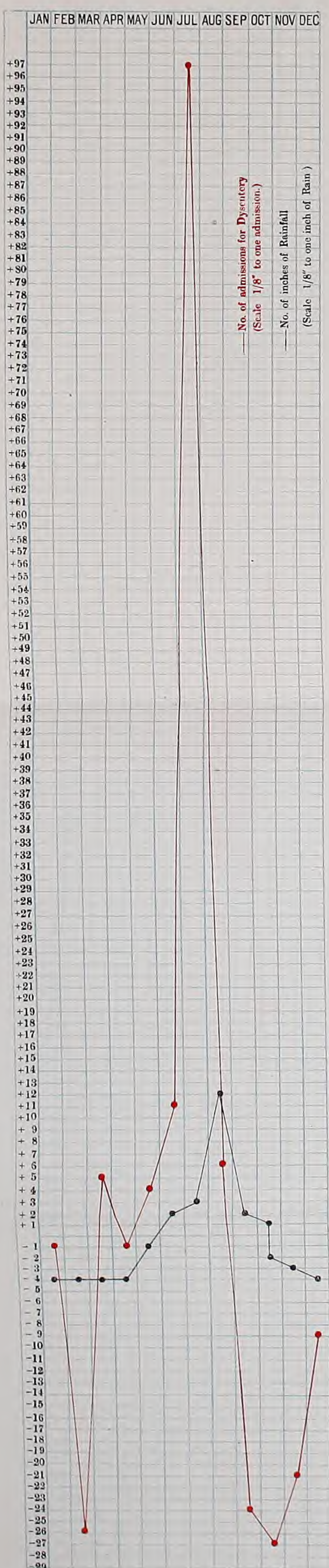
Chart II.



Mean of average Monthly admission rates
for Dysentery and Diarrhœa 32.
Mean of average monthly rainfall 5.

Charts representing the departure from the mean of the Monthly admission rates for Dysentery and Diarrhœa in relation to the rainfall in Hazaribagh Central Jail during the year 1910.

Chart III.



Mean of average Monthly admission rates for Dysentery and Diarrhœa 42

Mean of average monthly rainfall 4

The following table shows the admission rate under the heading of occupations:—

TABLE I.—*Showing the admissions per mille for dysentery and diarrhœa in relation to occupation during January-December 1910.*

	1	2	3	4	5	6	7	8	9	10	11	12
Name of occupation.	Cooks.	Sweepers.	Garden and agriculture.	Oil mill.	Aloe pounding.	Aloe net.	Aloe twine.	Cane and bamboo.	Durce factory.	Cloth factory.	Printing.	Bakery.
Total No. of admissions	6	4	11	9	63	3	53	1	13	6	2	3
Average daily strength.	13	30	28	9	56	5	40	5	33	30	5	4
No. of admissions per mille.	461	133	393	1,000	1,125	600	1,325	200	394	200	400	750

TABLE I.—*Showing the admissions per mille for dysentery and diarrhœa in relation to occupation during January-December 1910.*

	13	14	15	16	17	18	19	20	21	22	23	24
Name of occupation.	Blanket factory.	Surgical dressing factory.	Carpenter and blacksmith.	Tailors.	On miscellaneous jail service.	Employed in preparing articles for jail use or consumption.	Hospital (sick.)	C. G. and special gang.	Wall guards.	Convict overseers.	Under-trial prisoners.	Pumps.
Total No. of admissions	4	2	2	7	10	10	6	8	6	5	12	9
Average daily strength.	39	25	10	11	27	40	47	117	19	38	10	22
No. of admissions per mille.	102	80	200	636	370	250	128	67	315	131	1,200	409

The largest numbers of admissions occurred from among the convicts engaged in aloë-twine manufacture (1·325 per mille), and from among the under-trial prisoners (1,200 per mille).

The former class comprises mainly prisoners who are not in robust health and are unable for harder work; the latter are living under depressing conditions and without any occupation; these facts might account for an increased susceptibility to intestinal diseases.

It is noteworthy that those in closest contact with the sick showed no special liability to the disease; sweepers gave a comparatively low admission rate of 133 per mille while among the permanent hospital staff and the convict sick attendants, (the latter included under column 17), no case was reported. Drinking water for the jail is pumped from a tank near by, and is boiled and cooled before being distributed throughout the jail in pipes.

Even those prisoners who have freest access to unboiled water namely, the out-door gang, (columns 3 and 24 in the table), did not show a high admission rate.

Moreover these diseases were not prevalent to any extent in the Reformatory School, (28·4 cases per mille in 1910 as compared with 4,728 cases per mille in the jail during the same year), which is situated a few hundred yards from the jail and draws its water-supply from the same source.

Neither was there any evidence of infection through the prisoner's food which was all served out thoroughly cooked. The strictest measures for isolation and treatment of dysentery cases were systematically enforced by the Superintendent of the Jail.

II. Methods of Observation.

In investigating the causation of dysentery as it occurred in the jail, the following methods were adopted :—

(a) Laboratory examination of the stools.

The stools were passed into specially-constructed tin vessels provided with lids, the whole being sterilised before each issue. Specimens of the stools were examined as fresh as possible and were put through the following routine, as a rule, on six successive days :—

- (a) Naked-eye examination. The consistency, presence of faecal matter, mucus, blood and pus and the reaction to litmus paper were noted.
- (b) Microscopic examination. Under a one-sixth lens the presence of debris, of formed elements such as epithelium, leucocytes, red blood corpuscles, of worms, worm ova and protozoa was noted.

- (c) The stools were plated on Conradi-Drigalski medium; preferably a flake of mucus was selected for planting, after being washed in 3-4 changes of normal salt solution. The plates were examined, after incubation at about 37° C. for twenty-four hours, for the presence of "dysentery" colonies.
- (d) In a series of cases the stools were planted on Musgrave's medium also in order to determine whether amœbæ could be cultivated from them.

(b) Clinical examination of the cases.

Observation was especially directed to the following points:—(a) the temperature, (b) the condition of the tongue, (c) the frequency of the stools, (d) the presence of griping and tenesmus, (e) abdominal pain and tenderness, (f) pain, tenderness or enlargement of the liver.

The agglutinative power of the blood-serum on *Bacillus dysenteriae* ("Shiga" and "Flexner" types) was tested in many cases and in selected cases repeated blood-counts were made, including enumeration of the total red and white cells and differential leucocyte counts.

(c) Post-mortem examination.

Post-mortem examination was made in fatal cases, with microscopic and cultural examination of scrapings from the diseased intestine. Pieces of tissue were removed for embedding and section.

III. Results.

Preliminary remarks.

Altogether 268 cases of dysentery came under observation in the jail. In 14 (4·9 per cent.) of them a bacillus of the dysentery group was isolated; in 52 (19·5 per cent.) motile amœbæ were found microscopically; in 5 cases distinct evidence of tuberculosis of the intestine was found *post-mortem*.

In one of these tubercular cases motile amœbæ had been found in the fæces during life but no lesion of the large intestine was found *post-mortem* except patchy congestion and no amœbæ were detected microscopically in scrapings from the large and small intestines. In the remaining four cases neither bacilli nor amœbæ had been found.

In one of these cases the patient was under treatment for phthisis pulmonalis when first admitted for dysentery; of the others, three showed signs of active tuberculosis of the lungs on admission and one had intestinal symptoms

only. These four cases had from 3 to 6 previous admissions for dysentery and diarrhœa.

The intestinal symptoms were those of obstinate chronic dysentery.

Post-mortem, the lower part of the ileum in four cases showed characteristic tubercular ulcers, in one case, congestion only; caseating mesenteric glands were found in four cases.

In only one case was there ulceration of the large intestine, in the form of a few superficial erosions of the lower rectum without specific characters.

In one case there was general miliary tuberculosis; and in four cases there were tubercular foci in the lungs.

The following table gives an analysis of the further results of microscopic examination of the stools.

TABLE II.—*Showing the frequency of the occurrence of certain parasites and ova in the faeces.*

	Trichomonas intestinalis.	Balantidium coli.	Lambia intestinalis (?).	Ankylostoma ova in faeces or adults found post- mortem.	Ascaris ova.	Trichocephalus ova.	Oxyuris ova.	Strongyloides stercoralis.	Cestodes.
	1	2	3	4	5	6	7	8	9
Total in 268 cases.	184	1	1	92	13	2	4	5	1
Percentage .	68·6	0·35	0·35	34·3	5	0·7	1·4	1·8	0·35

(b) Characters of the stools in dysentery cases.

Apart from the finding of motile amœbæ microscopically, and the recovery of dysentery bacilli by culture, no characters were observed in the stools which might serve as a basis for classification of the cases into distinctly separate types. The features of the motions seem to depend upon the grade of severity and upon the duration of the case, rather than upon any specific action of the causal virus.

The stools, in the acute stages, generally consist of small quantities, (1-3 drachms), of tough muco-pus, streaked with bright red blood, the appearance sometimes being reminiscent of a phthisical sputum; or there may be an ounce or two of turbid abilious fluid mixed with masses, flakes or streaks of more or less blood-stained mucus. Stools of this kind may alternate with more copious loose fæculent motions containing mucus and blood.

As convalescence becomes established the fæcal element in the stools predominates, first fluid, then solid with more or less admixture of mucus.

In the chronic stages of the disease the motions often contain large amounts of tenacious blood-stained mucus, shreds of sloughy-looking material and blood-clots with more or less bile-stained sediment. The odour may be very offensive but does not seem to be in any way characteristic. The reaction of the stool is generally alkaline, seldom neutral or acid.

Microscopically the muco-sanguineous pus is found to consist of leucocytes mostly of the polymorphonuclear variety, red blood corpuscles and a small proportion of epithelium.

In stained preparations the appearance often resembles that of a film of abscess pus, many of the leucocytes containing coli-like bacilli.

The looser fæculent motions consist of bile-stained granular debris in which partially-digested vegetable cells and a large proportion of epithelium may generally be recognised.

The microscopic characters of the amœbæ and the results of cultivation of the stools will be described later.

(c) Clinical examination.

The cases do not seem to present any groups of features which might be definitely correlated with the laboratory findings, and the classical distinctions between the "bacillary" and "amoebic" types of dysentery were not observed clinically.

No group of cases occurred as a distinct epidemic; bacillary and amœbic cases as well as those of "uncertain origin" seemed to occur quite erratically. Some further facts from the collected records of these cases are given in table III.

TABLE III.—*Showing case-mortality, average duration, etc. (excluding tubercular cases).*

	Fatal cases.	Cases lasting over 60 days.	Average duration of remaining cases.	Cases showing one or more previous admissions to hospital for dysentery and diarrhoea.
Bacillary cases.	42·8 p. c.	25 p. c.	27 days.	42·8 p. c.
Amœbic cases .	11·7 p. c.	15·5 p. c.	26 „	50 p. c.
All other cases	10·4 p. c.	3·5 p. c.	22·7 „	34·2 p. c.

In most cases the disease ran an afebrile course. In neither bacillary nor amœbic cases was any rise of temperature recorded during the stay in hospital.

Of the remaining cases, in five the temperature was slightly raised (99°-100°) during the first day or two of illness.

In one case, (No. 263), the patient had been admitted to hospital for fever (temperature 102·4°) eight days previously to the onset of dysenteric symptoms.

In another case there was a paroxysmal rise to 103·8° on the evening of the second day of illness, the temperature being normal next morning and so remaining thereafter.

The tongue showed no characteristic change and was generally remarkably clean. In some cases there was a slight white coating, in others the tongue showed a red raw appearance without fissures or ulcers.

The stools generally numbered 2-5 during the 24 hours in mild cases and 10-20 in severe cases at the height of the disease; the largest daily number recorded in any case is 30. Gripping and tenesmus were not invariably present and were seldom severe.

There was often remarkable freedom from tenderness on pressure of the abdomen, even in severe cases which *post-mortem* showed extensive ulceration. When present, this symptom was generally most marked over the sigmoid flexure or the cœcum.

In the more chronic cases thickening of the colon could sometimes be made out on palpation.

In no case was there marked pain, tenderness or enlargement of the liver.

The bacillary cases showed entire freedom from complications.

Of the amoebic type of the disease œdema of the feet occurred in two cases, one of which ended fatally, while the other recovered. Among the cases "of uncertain origin" œdema of the feet occurred in five, all of them ultimately fatal.

In one chronic case (No. 23) the patient complained of migrating pains in the joints. After six days the pain became limited to the knee-joint from which it finally disappeared a week later. The pain in the joints was not accompanied by any appreciable swelling; its relation to the dysentery seems uncertain.

(d) **Blood examination.**

The following tables show the results of blood-counts made in some of the cases :—

TABLE IV.—*Blood-counts in bacillary cases.*

Case No.	Period of examination.	Red cells per c. mm.	White cells per c. mm.	Polynuclears p. c.	Lymphocytes p. c.	Large mononuclears. p. c.	Eosinophiles p. c.	REMARKS.
214	2nd day (10th admission)	3,950,000	9,800	66.2	25.8	7.3	0.7	Severe chronic case with much wasting: œdema of head, hands and feet. Tenderness of descending colon and sigmoid. Three injections 20 c. c. anti-dysenteric serum given at intervals of 2 and 5 days. Died. <i>Post-mortem</i> : large intestine œdematous and infiltrated; extensive ulcers; 2 ankylosoma duodenale found.
	65th day	..	25,600	74.6	23.0	1.6	0.8	
	67th "	..	44,780	69.3	28.2	1.3	0.2	
	69th "	..	18,920	56.7	39.2	3.9	0.2	
	71st "	..	25,720	77.1	21.8	0.7	0.4	
	73rd "	..	23,680	72.7	24.6	2.6	0.1	
	76th "	..	22,080	46.3	48.4	4.8	0.5	
260	3rd day	...	8,900	74.8	18.3	6.2	0.7	1st admission: mild case. Recovered.
	15th "	..	5,520	67.6	27.1	3.9	1.4	
262	2nd day	...	6,920	79.4	17.0	3.0	0.6	1st admission: mild case. Recovered.
264	5th day	...	7,660	58.3	38.0	3.3	0.4	1st admission: mild case. Recovered.

TABLE V.—*Blood-counts in anaemic cases.*

Case No.	Period of examination.	Red cells per c. m.m.	White cells per c. m.m.	Polynuclears p. c.	Lymphocytes p. c.	Large mononuclears p. c.	Eosinophiles p. c.	REMARKS.
35	2nd day	9,960	61.3	30.6	4.6	0.5	7th admission : mild case. Recovered.
141	2nd day . . 2 months later	9,720 6,200	61.9 75.2	33.8 21.4	2.7 3.2	1.6 0.2	1st admission : chronic, severe case. Recovered.
161	1st day . .	3,642,400	7,160	57.0	38.0	4.0	1.0	1st admission : moderately severe. Recovered. (oxyuris and ascaris ova).
239	5th day . . 37th " . .	4,387,500 4,563,333	7,900 11,600	62.6 69.9	24.1 22.9	9.9 4.7	3.4 2.5	2nd admission : moderately severe. Recovered.
243	15th day . . 3 months later .	4,562,000 ...	10,960 8,970	71.4 68.9	20.7 29.1	4.2 1.2	3.7 0.8	3rd admission : severe. Died. <i>Post-mortem</i> : extensive thickening and ulceration of colon.
244	10th day . .	3,714,700	16,580	74.3	14.4	6.0	5.3	1st admission : severe. Died. <i>Post-mortem</i> : congestion and ulceration colon. One ankylostome in duodenum.
245	15th day . .	4,919,203	12,320	48.5	37.0	10.0	4.5	4th admission : mild. Recovered.
250	1st day . . After 2 months	7,560 7,400	63.6 70.8	30.1 26.0	5.8 2.5	0.5 0.7	2nd admission : chronic. Recovered (ankylostoma ova).
251	2nd day	8,740	71.2	22.3	5.7	0.8	1st admission : mild. Recovered (ankylostoma ova).
255	4th day . . 8th "	11,000 11,400	78.6 66.5	17.8 30.5	2.6 2.7	1.0 0.3	2nd admission : moderately severe. Recovered (ankylostoma ova).

TABLE VI.—*Blood-counts in cases other than bacillary or anæmic.*

Case No.	Period of examination.	Red cells per c. m.m.	White cells per c. m.m.	Polynucleurs p. c.	Lymphocytes p. c.	Large mononuclears p. c.	Eosinophiles p. c.	REMARKS.
2	2nd day	...	11,140	60.1	33.7	5.1	1.1	2nd admission : mild. Recovered.
4	3rd day	3,158,350	5,140	59.2	35.3	5.0	0.5	2nd admission : chronic, severe. Died. <i>Post-mortem</i> : thickening, irregular ulcers of colon.
13	8,280	73.1	24.8	1.9	0.2	3rd admission : chronic, severe. Died. <i>Post-mortem</i> : extensive ulcers of colon (11 ankylostomes).
49	3,280 5,740	60.3 69.3	37.6 27.7	1.7 2.3	0.4 0.7	3rd admission. moderately severe. Recovered.
74	9,740	69.7	26.4	3.4	0.5	2nd admission : severe, chronic. Died. <i>Post-mortem</i> : extensive ulcers colon (ankylostomes).
123	1st day	5,065,000	10,000	65.5	27.0	4.5	3.0	1st admission : mild. Recovered
163	1st day readmission.	...	5,800	64.5	30.5	3.5	1.5	2nd admission : mild. Recovered. (ankylostoma ova).
197	1st day readmission.	...	6,840	66.4	26.0	5.8	1.8	2nd admission : mild. Recovered.
231	6th day readmission.	...	9,740	68.7	25.3	5.6	0.4	2nd admission : mild. Recovered (ankylostoma ova.)

TABLE VI.—Blood-counts in cases other than bacillary or amœbic—contd.

Case No.	Period of examination.	Red cells per c. m.m.	White cells per c. m.m.	Polynuclears p. c.	Lymphocytes p. c.	Large mononuclears p. c.	Eosinophils p. c.	REMARKS.
232	2nd day	4,380,000	8,200	53.0	29.0	10.4	7.6	2nd admission : mild. Recovered.
237	5th day	...	6,400	79.0	9.5	5.5	6.0	1st admission : mild. Recovered.
246	...	4,800,000	6,820	76.4	20.0	3.3	0.3	2nd admission : chronic, severe. Diel. <i>Post-mortem</i> congestion and old ulcers of colon (ankylostomes).
247	3rd day 4th " 6th "	4,856,000	9,620 8,380 7,240	59.5	34.3	5.2	1.0	3rd admission : moderate, severe. Tenderness right hypochondrium, temperature 103.8, 2nd day : normal ; thereafter liver not enlarged. Recovered (ankylostoma ova).
249	2nd day	4,442,300	5,800	69.0	24.0	6.1	0.9	10th admission : mild. Recovered.
253	2nd day 3rd " 5th " ...	4,739,130	14,180 11,080 11,580 10,980	57.1 65.2 64.5 61.9	33.7 30.4 28.5 32.1	3.2 3.4 5.0 4.7	1.1 1.0 2.0 1.3	4th admission : mild, tenderness hypogastrium, no enlargement of liver. Recovered.
254	1st day	7,120 6,640	54.3 52.1	36.2 39.1	5.9 7.1	3.6 1.7	2nd admission : mild. Recovered (ankylostoma ova).

TABLE VI.—*Blood-counts in cases other than bacillary or amæbic. —concl'd.*

Case No.	Period of examination.	Red cells per c. m.m.	White cells per c. m.m.	Polynuclears p. c.	Lymphocytes p. c.	Large mononuclears p. c.	Eosinophiles p. c.	REMARKS.
256	4th day	...	22,680	82.8	12.4	4.25	0.6	1st admission: mild, tenderness in hypogastrium, no enlargement of liver. Recovered.
	5th "	...	13,280	
	6th "	...	16,500	
	7th "	...	11,340	71.0	21.8	6.8	0.4	
257	5th day	...	8,660	65.3	32.5	2.0	0.2	1st admission: mild. Recovered (ankylostoma ova).
259	6th day	...	8,140	77.5	15.3	6.0	1.2	2nd admission: mild. Recovered.
261	3rd day	..	12,980	69.8	26.4	3.2	0.6	4th admission: mild. Recovered (ankylostoma ova).
	11th day	...	7,000	58.8	37.2	3.8	0.2	2nd admission: mild. Recovered (ankylostoma ova).
266	4th day	...	6,320	68.0	28.1	2.6	1.3	2nd admission: mild. Recovered.
268	2nd day	...	6,980	7th admission: mild. Recovered.

It is noticeable that in some apparently uncomplicated cases of dysentery a high and sustained leucocytosis may occur, although this is not the rule; this is in agreement with the findings during the dysentery enquiry in Bombay.

(e) Agglutination reaction.

The following table shows the results of the agglutination test in bacillary cases :—

TABLE NO. VII.—*Agglutination reaction in bacillary cases.*

Case.	Period of disease.	Reaction on B. Shiga with dilution.	Reaction on B. Flexner with dilution.	Reaction on own bacillus with dilution.	REMARKS.
25	9th day	1 in 20 ±
	12th „	1 in 10 —	1 in 10 —	1 in 20 ±
27	5th day	1 in 10 —
	12th „	1 in 10 —
29	Blood not examined. Patient, an epileptic suffering from mild dysentery, died during a seizure on day of admission.
34	4th day	1 in 80 +
	12th „	1 in 40 +
41	6th day	1 in 80 +
	12th „	1 in 10 +
115	26th day	1 in 80 +
128	Blood not examined. Bacillus recovered <i>post-mortem</i> only.
162	5th day	1 in 10 —	1 in 10 —	1 in 10 —	Mild case released from jail on 5th day.
198	9th day	1 in 10 +
	12th „	1 in 40 +

TABLE NO. VII.—*Agglutination reaction in bacillary cases—contd.*

Case.	Period of disease.	Reaction on B. Shiga with dilution.	Reaction on B. Flexner with dilution.	Reaction on own bacillus with dilution.	REMARKS.
206	14th day	1 in 10 —	1 in 10 —	1 in 10 —
214	13th day	1 in 40 +	1 in 10 —	1 in 10 —
	2 months later.	1 in 10 —	1 in 10 —	1 in 10 —	Recurrence.
260	4th day	1 in 10 —	1 in 10 +	1 in 20 +
	10th „	1 in 20 +
	14th „	1 in 10 —	1 in 10 +
262	1st day	1 in 10 —	1 in 10 —
	10th „	1 in 10 —	1 in 10 —	1 in 10 —
264	2nd day	1 in 10 —	1 in 10 —
	9th „	1 in 10 —	1 in 10 +	1 in 10 —

It is here apparent that, neither with stock cultures of B. Shiga and B. Flexner, nor with the autogenous bacillus is a reaction obtained in any cases with high dilutions of the serum.

Moreover, the agglutination test with stock cultures of “Shiga” and “Flexner” strains was applied in 130 cases of dysentery in which no dysentery bacilli were ultimately found and also in the cases of 40 prisoners who gave no history of dysentery. The results are shown in the following tables :—

TABLE NO. VIII.—*Table showing the agglutination reaction on B. Shiga and B. Flexner in 130 cases of dysentery from which no dysentery bacillus could be isolated.*

Maximum dilution in which the blood-serum agglutinated.	1 in 320	1 in 160	1 in 80	1 in 40	1 in 20	1 in 10	Negative 1 in 10.
(i) B. Shiga	1	0	4	1	8	15	101
(ii) B. Flexner	1	0	5	2	10	13	99

TABLE NO. IX.—*Table showing the agglutination reaction on (i) B. Shiga and (ii) B. Flexner in 40 cases of prisoners who gave no history of dysentery.*

Maximum dilution in which the blood-serum agglutinated.	1 in 80	1 in 40	1 in 20	1 in 10	Negative 1 in 10.
(i) B. Shiga	2	4	7	3	24
(ii) B. Flexner	0	3	5	4	28

Thus the evidence from these cases, as far as it goes, is unfavourable to the utility of the agglutination reaction as an aid in the diagnosis of bacillary dysentery.

(f) Post-mortem examination.

Large intestine.—In all the fatal cases the large intestine showed marked congestion and there was generally considerable pulpy gelatinous thickening of the submucosa. In a few instances, however, there was marked thinning and atrophy of the intestinal wall, little but the serous coat being left in parts.

In some cases the surface of the mucosa was covered with a more or less extensive diphtheritic exudate; this was a marked feature in all of the six fatal bacillary cases, neither did any of these bacillary cases show a state of advanced ulceration.

In the cases generally, congestion varied from a mild hyperæmia to the most intense injection with hæmorrhages, sometimes small and stellate or punctate, sometimes forming extensive uniform dark, blue or black areas of extravasation.

In many cases raised bluish gelatinous areas, oval or hemispherical in shape, were found projecting from the mucous membrane, varying in size from a pea to a large bean. Some of these presented single or multiple perforations at their apices through which a reddish or yellowish granular semi-fluid material could be expressed.

Ulceration of the most varied types was found, small irregular superficial erosions, areas of fine serpiginous ulceration or deeper ulcers with sloughy bases, extending down to the muscularis or even to the serous coat; some had perpendicular, some undermined edges.

The cæcum and rectum were perhaps the favourite sites, but the ulcers were not generally localised to a particular spot, and in many cases the whole extent of the large intestine was covered with ulcers, old and recent.

In two cases, multiple spherical bodies with short pedicles and varying in size from a pea to a marble, were found projecting from the mucous membrane

of the rectum. One of these cases was "amœbic," the other was "of uncertain origin."

The small intestine.—There was generally, in all cases of dysentery examined *post-mortem*, congestion of the lower part of the ileum, occasionally with sub-mucous hæmorrhages; the Peyer's patches were not especially involved.

In contrast to the tubercular cases, these dysentery cases showed no ulceration of the ileum except in one instance, where there was slight superficial erosion, confined to the lowermost four inches.

In some cases there was distinct atrophy of the wall of the small intestine.

The number of ankylostoma worms found *post-mortem* in any single case was generally 1-5 and never exceeded 20. In no case could serious damage of the gut be directly attributed to their presence.

The mesenteric glands.—The mesocolic glands generally showed more or less distinct enlargement; they were, as a rule, uniformly soft in consistence and pale pink in colour; in a few cases they showed deep congestion with stellate capsular hæmorrhages and were plum-coloured on section.

In four cases, all "of uncertain origin," the liver was slightly enlarged and fatty. The lungs in two cases, both "of uncertain origin," showed infarctions, while in another case there was gangrene of the lung.

Otherwise there was a remarkable absence of complications.

In none of the six fatal amœbic cases could any amœbæ be detected in stained paraffin sections of the ulcers. A detailed microscopic study of the pathological material has not yet been completed.

(g) Bacilli of the dysentery group isolated.

In fourteen cases organisms were isolated whose ultimate reactions would seem to establish their claim to inclusion within the group of the bacillus dysenteriae.

The differentiation of bacilli by means of their actions in fermenting sugars presents, perhaps, peculiar difficulties in a warm climate, but in the following description only such results of sugar fermentation experiments are included as have been verified by repeated observations in each case.

All these bacilli have the following points in common. (i) On Conradi-Drigalski medium they form fine transparent blue colonies (*i.e.* they do not form acid in lactose medium). (ii) Microscopically they are found to be short rods, showing, as a rule, very active Brownian movement, without definite progressive motility. (iii) They stain easily with methylene-blue or other aniline dye but are Gram-negative. (iv) A transplant on an agar slope shows a fine white filmy moist growth after 24 hours. (v) They form acid, but no gas in glucose media.

Their further characteristics are set forth in the following table :—

TABLE X.—*Showing distinctive reactions of the dysentery bacilli isolated.*

Case No.	Sitmas milk.	Dextrin.	Maltose.	Mannite.	Indol.	Effects on rabbits.
25	Slight acid after 24 hours, then alkaline.	No change.	Slight acid.	Acid.	+ after 7 days.	2 c. c. of a 24 hours' broth culture intraperitoneally. Diarrhœa with blood and mucus. Paralysis of hind legs. Death in two days. <i>Post-mortem</i> : acute inflammation of colon.
27	No change . . .	Slight acid.	No change.	Acid.	+ after 7 days.	Half of a 24 hours' growth on agar slope given intraperitoneally. Diarrhœa (blood and mucus). Paralysis of hind legs. Death after 24 hours. <i>Post-mortem</i> : acute inflammation of colon.
29	No change . . .	No change.	Acid.	Acid.	+ after 7 days.	Half of a 24 hours' agar growth intraperitoneally. Severe diarrhœa (mucus) and wasting. Recovery.
34	Slight acid after 24 hours, then alkaline.	Acid.	Acid.	Acid.	+ after 7 days.	Half of a 24 hours' agar growth intraperitoneally. Diarrhœa (mucus and blood). Death after 24 hours. <i>Post-mortem</i> : inflammation of colon.
41	No change . . .	No change.	Slight acid.	No change.	—	3 c. c. of a 24 hours' broth culture intraperitoneally. Severe diarrhœa (mucus and blood). Wasting; recovery after 5 days.
115	Slight acid after 24 hours, then alkaline.	No change.	Slight acid.	No change.	—	Half of a 24 hours' agar growth intraperitoneally. No diarrhœa. Death within 24 hours. <i>Post-mortem</i> : congestion of colon.
128	No change . . .	Slight acid.	Acid.	Acid.	+ after 10 days.	3 c. c. of a 24 hours' broth culture intraperitoneally. Paralysis of hind legs; diarrhœa. Death after 36 hours. <i>Post-mortem</i> : extreme congestion of peritoneum.

TABLE X.—*Showing distinctive reactions of the dysentery bacilli isolated—contd.*

Case No.	Sitmas milk.	Dextrin.	Maltose.	Mannite.	Indol.	Effects on rabbits.
162	Slight acid after 24 hours, then alkaline.	No change.	Acid.	No change.	—
198	Slight acid after 24 hours, then alkaline.	No change.	Acid.	Acid.	+ after 10 days.	4 c. c. of a 48 hours' broth culture given intraperitoneally. Diarrhoea; death in 24 hours. <i>Post-mortem</i> : large intestine inflamed, extreme congestion of abdominal vessels.
206	Slight acid after 24 hours, then alkaline.	No change.	No change.	No change.	—	4 c. c. of a 48 hours' broth culture given intraperitoneally. Diarrhoea; death within 24 hours. <i>Post-mortem</i> : colon inflamed, lymphoflakes on mucosa.
214	Slight acid after 24 hours, then alkaline.	No change.	Slight acid.	Acid.	+ after 10 days.	4 c. c. of a 48 hours' broth culture intraperitoneally. Diarrhoea, paralysis (?). Death within 24 hours. <i>Post-mortem</i> : colon inflamed, abdominal vessels congested.
260	Slight acid after 24 hours, then alkaline.	No change.	Slight acid.	Acid.	+ after 10 days.	3 c. c. of a 24 hours' broth culture intraperitoneally. Paralysis, diarrhoea; death in 24 hours. <i>Post-mortem</i> : submucous hæmorrhages in colon and in bladder. Injection of peritonæum.
262	Slight acid after 24 hours, then alkaline.	No change.	Acid.	Acid.	+ after 10 days.	3 c. c. of a 24 hours' broth culture intraperitoneally. Diarrhoea, (mucus), weakness and emaciation. Died on 6th day, submucous hæmorrhages in cecum; injection of peritonæum and of bladder and tunica vaginalis.
264	Slight acid after 24 hours, then alkaline.	No change.	No change.	No change.	+ after 10 days.	3 c. c. of a 24 hours' broth culture intraperitoneally. Diarrhoea (mucus blood). Wasting, death on 8th day. <i>Post-mortem</i> : congestion of colon. Peritoneal injection.

The differences in the reactions of these different bacilli are very striking and are hard of interpretation. The question as to whether such differences depend upon mutations in the characters of one single species or are in themselves genuinely specific, still remains to be settled.

In any case it would seem that this type of bacillary infection is not highly contagious.

These bacilli were isolated during the acute phases of the disease only and repeated attempts to recover the bacillus from the stools of the cases during convalescence and, later, while in the "post-dysenteric gang," met with no success.

In none of the five fatal cases in which bacilli had been cultivated from the stools during life were they recovered by cultivation from scrapings from the ulcerated intestine made *post-mortem*. In one of these cases autopsy was made within an hour of death.

On the other hand, in the single case (No. 128), in which dysentery bacilli were cultivated from the scraping of an ulcer, the *post-mortem* had not been made until 11 hours after death.

(h) The amœbæ met with.

Cultivation of amœbæ.—During the work of the dysentery enquiry in Bombay it had been observed that amœbæ could, in a large proportion of experiments, be cultivated from a great variety of sources, fæces, both dysenteric and healthy, among others.

This experience was repeated at Hazaribagh; in a series of 76 cases of dysenteric stools planted on Musgrave's medium, in 74 were amœbæ found growing. Moreover, amœbæ, apparently similar in type, were cultivated from healthy stools and from samples of the jail water, taken both before and after boiling.

In cultures from all these different sources it was noted that amœbæ were often found growing in association with colonies of moulds and other contaminating organisms from the air.

It was found, moreover, that uninoculated plates of Musgrave's medium generally showed colonies of air organisms on their surface after standing for two or three days at room temperature.

On examination of such colonies under the low power of the microscope, it became evident that on some occasions amœbæ were growing in association with these self-sown bacteria from the air.

In a series of experiments, details of which are given in the following table, it was found that in 14 out of 36 such uninoculated plates, amœbæ could be demonstrated.

TABLE XI.

Serial No.	Date.	Conditions of experiment.	Presence of amœbæ.	REMARKS.
1	12th August 1910.	Uninoculated Petri plate left out on laboratory table without preliminary exposure to air.	+18th August 1910.	Cysts of types (a) and (b).
2	12th August 1910.	Same as above	—Up to 5th September.	Medium overgrown by moulds.
3	12th August 1910.	Uninoculated Petri plate left out on laboratory table without preliminary exposure to air.	—Up to 5th September.	Ditto ditto.
4	18th August 1910.	Plate first opened exposed to the air for 5 minutes then left out on laboratory table as before.	—Up to 11th September.	Ditto ditto.
5	18th August 1910.	Similar to above but preliminary exposure to air 10 minutes.	—Up to 5th September.	Ditto ditto.
6	18th August 1910.	Similar to above but preliminary exposure to air 20 minutes.	—Up to 5th September.	Ditto ditto.
7	18th August 1910.	Similar to above but preliminary exposure to air 30 minutes.	—Up to 5th September.	Ditto ditto.
8	18th August 1910.	Similar to above but preliminary exposure to air 1 hour.	—Up to 5th September.	Ditto ditto.
9	18th August 1910.	Similar to above but preliminary exposure to air 1½ hours.	+22nd August	Ditto ditto.
10	18th August 1910.	Similar to above but preliminary exposure to air 2 hours.	—Up to 5th September.	Medium dried up.
11	18th August 1910.	Plate left out on window-sill of hospital dysentery ward without preliminary exposure to air.	—Up to 9th September.	Medium dried overgrown by moulds.
12	18th August 1910.	Similar to above	—Up to 6th September.	Ditto ditto.
13	18th August 1910.	Plate left out on window-sill of hospital general ward without preliminary exposure to air.	+23rd August	Cysts of type (a) and (b).
14	18th August 1910.	Similar to above	+28th August	Ditto ditto.
15	18th August 1910.	Plate left out on table of hospital office without preliminary exposure to air.	+22nd August	Ditto ditto.
16	18th August 1910.	Plate left out on floor of "post dysenteric gang" ward without preliminary exposure to air.	—Up to 11th September.	Medium overgrown by moulds.
17	18th August 1910.	Plate left out on floor of No. 1 jail ward without preliminary exposure to air.	+30th August	Cysts of type (a) and (b).
18	18th August 1910.	Plate left out in central tower of jail without preliminary exposure to air.	--Up to 9th September.	Medium dried up.

TABLE XI—contd.

Serial No.	Date.	Conditions of experiment.	Presence of amoebæ.	REMARKS.
19	18th August 1910.	Plate left out on window ledge of tank room without preliminary exposure to air.	—Up to 6th September.	Medium overgrown by moulds.
20	18th August 1910.	Plate left out on table of jail office without preliminary exposure to air.	—Up to 6th September.	
21	12th December 1910.	Uninoculated Petri plate left out on laboratory table without preliminary exposure to the air.	+3rd January 1911.	Cysts of types (a) and (b).
22	12th December 1910.	Petri plate first opened and exposed to the air for 5 minutes, then left out on laboratory table as before.	—Up to 3rd February 1911.	Medium dried.
23	12th December 1910.	Similar to above but preliminary exposure to air 10 minutes.	—Up to 3rd February 1911.	Ditto.
24	12th December 1910.	Similar to above but preliminary exposure to air, 20 minutes.	+3rd January 1911.	Cysts of type (a) and (b).
25	12th December 1910.	Similar to above but preliminary exposure to 30 minutes.	+3rd January 1911.	Ditto ditto.
26	12th December 1910.	Similar to above but preliminary exposure to air 1 hour.	+3rd January 1911.	Ditto ditto.
27	12th December 1910.	Similar to above but preliminary exposure to air 1½ hours.	+3rd January 1911.	Ditto ditto.
28	12th December 1910.	Similar to above but preliminary exposure to air, 2 hours.	+13th January 1911.	Ditto ditto.
29	12th December 1910.	Petri plate left out on window-sill in hospital.	+3rd January 1911.	Ditto ditto.
30	12th December 1910.	Petri plate left out on window-sill in hospital general ward, without preliminary exposure to air.	—Up to 3rd February 1911.	Medium dried.
31	12th December 1910.	Petri plate left out on table in hospital office without preliminary exposure to air.	+6th January 1911.	Cysts of type (a) and (b).
32	12th December 1910.	Petri plate left out on floor of "post-dysenteric gang" ward without preliminary exposure to air.	—Up to 3rd February 1911.	Medium dried.
33	12th December 1910.	Petri plate left out on floor of No. 1 jail ward without preliminary exposure to air.	—Up to 3rd January 1911.	Ditto.
34	12th December 1910.	Petri plate left out on ledge near top of central tower of jail, without preliminary exposure to air.	—Up to 3rd January 1911.	Ditto.
35	12th December 1910.	Petri plate left out on window ledge of tank room without preliminary exposure to air.	—Up to 3rd January 1911.	Ditto.
36	12th December 1910.	Plate left out on table of Superintendent's office without previous exposure to air.	—Up to 3rd January 1911.	Ditto.

Subcultures of these amœbæ were readily obtained by taking a loopful of the mixed growth of amœbæ, either vegetative forms or cysts, and bacteria, and stroking it on the surface of a fresh plate of Musgrave's medium.

The subcultures were generally kept in a moist atmosphere at a temperature of 22°-28° C. for the first day or two: the optimum temperature for their multiplication probably lies within these limits.

Generally, after from 12 hours to two days, there is an abundant crop of motile amœbæ along the needle-stroke of inoculation; as a rule, after 5-6 days many of the amœbæ are found to have become encysted.

In order to obtain a culture which would certainly represent only one species of amœbæ, the following method was adopted.

A loopful of growth is inoculated at one point of the plate; thence a radiating stroke is produced on the surface of the medium with a fine platinum needle. On examining this stroke under the low power several amœbæ are generally found at the end of it. From this point a second stroke is made, and so on, until, finally, one is selected which contains only a single amœba towards its extremity: this single amœba is then cut off from the rest of the growth by a stroke of carbolised vaseline painted on the surface of the medium after the method of Walker²⁵.

Pure cultures thus obtained were most easily preserved from contamination by subculturing on test-tube "slopes" of Musgrave's agar.

With a Zeiss A A lens and a No. 12 eye-piece the main processes of the life-cycle may be roughly followed on the inverted Petri plates, but, for examination under the higher powers of the microscope, the following method of subculture was employed.

A slab of Musgrave's agar, half an inch square, is cut out of a fresh plate of the medium with a sterile knife and placed on a sterile slide.

A loopful is then taken from an old culture containing cysts and stroked on the surface of the agar square; a sterile cover glass is placed on top and its edges are sealed with melted paraffin wax. An air space should be left between the edges of the agar slab and the paraffin wall.

Thus a microscopic moist chamber is provided, sealed against external contamination. Such a preparation may be kept in a warm microscope chamber at a temperature of 25° C. Examining such a slide-culture under the low power, one picks out a spot towards the tail end of the inoculation stroke where the cysts are thinly scattered; a single one is then isolated under the oil-immersion lens for continuous observation.

For permanent preparations, fixation after the method of V. Wasielewski and Hirschfeld²⁶ gave the best results.

The principal fixatives used were Osmic acid (2 per cent.) and corrosive-

acetic-alcohol, followed by Giemsa's stain and Iron-haematoxylin-Eosin, respectively.

The first method of fixation gives the best picture of the plasma, the second brings out the nuclear material particularly well.

MORPHOLOGY AND LIFE CYCLE OF THE CULTIVATED AMOEBÆ.

A reference to table XI shows that, in 12 out of the 14 growths of amoebæ obtained from the air in the manner described two types of cysts were distinguished.

The following description is concerned only with the growths of amoebæ occurring on the single plate. (No. 24 in the table.)

Cysts of type (*a*) (Fig. 1) have a diameter of 8-14, generally 10-12, micra; their shape is round, oval, more frequently trihedral, polygonal or stellate; the wall is about 0.25 micra in thickness and has a strongly marked double contour; the outer layer is generally wrinkled, reminding one of the outline of an *Ascaris* ovum.

The plasma is compact and coarsely granular and a circular or oval eccentric nucleus is as a rule, more or less distinctly visible in unstained specimens.

Cysts of type (*b*) (Fig. 19), on the other hand, are much smaller, having a diameter of 3-8 micra; the shape is round or oval, with little or no tendency towards the modified forms often assumed by cysts of type (*a*); the surface is smooth and the wall delicate with a faint double contour. The plasma is hyaline with thinly-scattered highly-refractile granules; a nucleus is hardly to be distinguished.

Cysts of both types stain an intense purple black with Iron-haematoxylin.

In order to determine whether these two well-defined types of cysts represent two different species or simply polymorphic forms belonging to a single species attempts were made to obtain cultures originating from a single cyst of each type by Walker's method as above described.

In many cases, however, the cysts isolated failed to germinate; it was therefore found more practicable to select a single motile individual as the starting-point of a strain.

(1) Amoebæ of type (*a*).

In this way cultures of amoebæ were obtained which were found to form cysts of type (*a*) only.

If such a culture be allowed to encyst, the cysts be transferred to a slide preparation, as above described, and one of them be isolated under the 1-12 lens, its development may be followed in detail.

After 1-3 hours (at a temperature of 22°—28°) a faint streaming movement of the contained granules becomes evident and a bright vacuole appears in the plasma. After a very variable period, from 10 min.—35 min., the vacuole suddenly shuts and either disappears entirely or leaves only a minute dark speck to mark its site; then, very slowly and gradually, it re-appears. This process is repeated at diminishing intervals until the vacuole may contract after irregular periods of 40-180 seconds the streaming of the granules becoming more and more active and the nucleus more clearly apparent in the meantime.

After 3-7 hours, as a rule, from the time of insemination, during active movements of the contents, a small knob of protoplasm is seen to thrust suddenly through the cyst wall at one point (Fig. 2.)

Streaming through the narrow outlet, the active amœba rapidly increases in size, so that a considerable portion of it may escape without any coincident shrinking of the contained part away from the inner wall of the cyst.

The amœba may become completely clear of the cyst within 5-20 minutes, of the rupture, and may by that time have reached a diameter of 15-25 micra.

The nucleus stands out distinctly as a circular or oval body with a dark centre and a clear peripheral halo; the total diameter is about 2·5 micra.

One contractile vacuole is invariably present which, at this stage, generally contracts, somewhat irregularly, once in 20-80 seconds and there may, more rarely, be two or more; clear circular areas about 1-2 micra in diameter, which remain uncontracted, are often seen in the plasma.

Sometimes the distinction between ectoplasm and endoplasm is remarkably clear, but often the protoplasm is uniformly granular. In this respect the same amœba shows varying characters at different times.

The pseudopodia vary much in form, may be broad and blunt or spinous simple or branched; the commonest form seems to be a single, rather broad blunt process, the margin of which is fringed with short spikelets. Several pseudopodia may be protruded in different directions at the same time; many bacteria may be ingested. In specimens fixed with Osmic acid and stained by Giemsa's solution the blunter pseudopodia are well demonstrated (Fig. 14).

After its escape from the cyst the amœba continues to move more or less actively over the surface of the fresh medium, leaving a trail of bacteria in its wake.

Actively motile amœbæ of this type have been watched on twelve occasions for periods of from 1-6 hours without showing any radical change in their behaviour.

On the other hand, on nine occasions an amœba under examination was observed to shrink and assume a rounded or oval form (Fig. 6), then the body undergoes an hour-glass constriction and finally divides into two daughter

amœbæ, each approximately half the size of the parent (Figs. 7 and 8); the whole process from the first evidence of constriction till complete division takes about two minutes.

The process of nuclear division was not followed in fresh specimens; indeed I have found the behaviour of these amœbæ, while undergoing fission, to be in this respect similar to that observed by Liston in one strain of amœbæ isolated by him from liver abscess pus. As in the amœbæ described by Liston, on the condensation of the plasma previous to division, the nucleus becomes obscure, nor in the new daughter amœbæ is a nucleus immediately to be seen; it takes shape gradually and not till 2-5 minutes after division does it become clearly visible.

In stained specimens some amœbæ were seen in which the nucleus, while taking the stain deeply, showed an appearance suggestive of simple fission (Fig. 19); in none was evidence of karyokinesis seen. In one stained specimen (Fig. 15), an amœba was seen showing an hour-glass constriction in the middle with a clear vacuole at each pole without distinct nuclear staining.

In fresh specimens, on many occasions, a small portion of a motile amœbæ was seen to become detached; these fragments showed no active movement and ultimately disappeared from view after half an hour or more. On four occasions, however, true motile buds, 2-5 micra in diameter, were seen to emerge from the parent body.

In specimens fixed by corrosive-alcohol-acetic acid and stained by Iron-hæmatoxylin, an amœba is often seen to contain 1 to 12 or more clear rounded spaces, 1 to 4 micra in diameter, with or without a central or eccentric dot which takes the chromatin stain faintly (Figs. 16, 17 and 18); these apparently correspond to the "internal buds," or "merozoites" of Noc.

Many small protoplasmic masses, one or more micra in diameter, some containing a distinct nucleus, others without any chromatin staining, are also seen in stained films from the cultures, sometimes collected into groups.

The small bodies without any visible chromatin possibly represent the detached fragments of protoplasm seen in fresh specimens.

It is, therefore, evident that multiplication by unequal budding also takes place in these amœbæ.

A third striking modification in the activity is very commonly seen in these cultures.

An amœba may throw out highly refractile globular or blunt branched motile processes, 2-4 micra in diameter, at one or more points on its surface. The whole of the original body may in a few minutes be completely overlaid by or absorbed in these amœboid excrescences which adhere to one another, the original nucleus being entirely lost to view.

Thus a writhing lobulated mass is produced which may assume the most bizarre forms, moruloid, moniliform or cochleate; the lobules composing it, some of which often show contractile vacuoles, may lie one on top of the other, so that all cannot be focussed at once (Figs. 11-13).

This process has been noted to occur in more than twelve cases while individual amœbæ were under observation.

Such bodies have been consecutively observed under the higher powers of the microscope on twenty recorded occasions with the following results:—

- (i) In four cases the lobulate body has moved about for periods of from 35 minutes to 5 hours and changed its shape continuously but has not undergone any alteration in character.
- (ii) In eleven cases one of the elements composing the mass has been seen to absorb the others into itself, gradually acquiring a visible nucleus, until an amœba of the ordinary vegetative type resulted.

This amœba was observed (a) in three cases, to retain its characters unaltered for periods up to 2 hours; (b) in 5 cases, to reassume the lobulate form after periods of from 3 minutes to 1½ hours; (c) in one case, to give off a bud, 5 minutes after formation; (d) also, in one case, to divide into two, 3 hours 15 minutes after formation.

- (iii) In two cases the lobulate body has been observed to divide into two similar masses.
- (iv) In three cases one of the processes has been observed to become detached, acquiring a visible nucleus and all the characters of the usual vegetative type. No forms definitely corresponding to these lobulate bodies have been found in stained specimens.

The significance of this form of activity is not clear; it may perhaps represent either a modification of the process of budding or a third distinct method of reproduction by multiple division.*

* Major Liston informs me that he has observed similar appearances in his cultures; he attributes them to the fact that the amœbæ concerned "are simply penetrating the surface of the agar." He points out that amœbæ grow below the surface of the agar, and that, in old cultures, cysts may be focussed in various planes of the medium.

I find that this is apparently a constant phenomenon in cultures of type (a).

It is very difficult to interpret the active movements of these burrowing amœbæ, as different points of their surface are at different levels, so that the whole body cannot be focussed at one time. Moreover, another amœbæ might suddenly come into view in the field of the microscope, having entered it from a higher or lower plane, and thus give rise to an appearance of division on the part of the amœba originally under observation.

It seems at least probable that the activities of these lobulate bodies here described may be due to modified processes of fission and budding going on below the surface of the agar.

The fact that these forms occur only below the surface accounts for their absence in fixed and stained films.

Three or four days after insemination some of the amœbæ generally become encysted.

Encystment seems often to be immediately preceded by remarkably active multiplication: this results in the formation of clumps of amœbæ which may become massed together, giving the appearance of plasmodia.

In the end each amœba becomes rounded, the body shrinks, becomes more granular in appearance and acquires a thin envelope; this is at first single but later presents the characteristic appearance of double contour, the outer layer being wrinkled. The original circular outline may be modified to become trihedral, polygonal or stellate, the nucleus becomes less distinct, the contractile vacuole ceases to open, until it becomes apparent that the youngest generation has reached the cystic stage from which its ancestors set out.

In stained specimens many of the amœbæ undergoing encystment are seen to contain numerous internal buds (Fig. 18).

Not all the amœbæ encyst about the same time; indeed many are often found still motile in cultures more than a month old.

(2) Amœba of type (b).

Attempts to obtain a culture the progeny of a single individual of this type did not succeed, but ultimately a culture originating from a number of small motile forms was obtained which, in subculture, was invariably found to yield cysts of the small type only.

Germination of the cysts has not been directly observed in this strain; on six occasions a single cyst on fresh medium was kept under the oil-immersion lens for from 10 hours to 3 days without showing any signs of germination.

Empty cyst shells have not been seen in unstained specimens, but in stained films from the cultures they appear as rounded shrivelled bodies about 3-5 micra in diameter. With Giemsa's stain they take a brownish or greenish colour, with Iron-hæmatoxylin they are light yellow.

The vegetative amœbæ of this type seldom measure more than 12 micra. Many small forms are also found, measuring as little as 2 micra.

The vegetative form (Fig. 21), is generally very filmy and delicate, showing a faint hyaline plasma, containing scattered granules without any definite distinction between ectoplasm and endoplasm. Pseudopodia are commonly single and rather broad and blunt; spinous pseudopodia were not seen in this form.

The nucleus is generally hard to distinguish in unstained specimens but may appear as a faint pinkish circular area 1-2 micra in diameter. One contractile vacuole is generally present in the active amœba and contracts once in 25-70 seconds.

Movement is, as a rule, more active than in type (*a*), the whole body gliding rapidly, often without any appreciable formation of pseudopodia.

Fission has been observed in fresh specimens 24 times (Figs. 22 and 23).

The amœba does not lose its motility and become condensed before fission as in type (*a*) but, while actively motile, it suddenly assumes a dumb-bell shape and divides into two daughter-amœbæ within one minute.

The formation and detachment of a bud about 1-4 micra in diameter has been observed in fresh specimens on four occasions.

Nothing has been observed in this strain approaching the formation of the lobulate bodies which are so characteristic in cultures of type (*a*), but, on the other hand, the following curious modifications of activity have been noticed.

An active amœba may suddenly become constricted in the middle, the two halves separating and giving a transitory appearance of fission; they, however, immediately come together again, the amœba resuming its former habit.

In the same way a small portion of the amœba may appear to separate off from the main body to become immediately reabsorbed.

These appearances are suggestive of abortive fission and gemmation, respectively.

On two occasions in cultures of this type, a motile amœba has been observed to become spherical and acquire a double-contoured envelope. One of them was a daughter amœba, the product of fission one hour old when encystment commenced; in the other case the amœba had given off a bud 33 minutes before.

In stained specimens (Fig. 25) the vegetative forms have a diameter of 2-14 micra; the nucleus, which in a well-grown form measures about 2 micra, stains less intensely than in the amœba of type (*a*); Osmic acid fixation followed by Giemsa staining fails to show a clear distinction between ectoplasm and endoplasm.

Fission forms (Fig. 27) and forms containing endogenous buds (Fig. 26) are seen, as also vacuolated bodies without any well-defined nucleus but with more or less scattered irregular chromatin masses or granules.

The details of the life-cycle of these two types of amœbæ have been studied in cultures on solid agar only; however, both types "*(a)*" and "*(b)*" have been found to multiply to some extent in diluted broth (1 in 10, 1 in 100), though this medium does not seem to be at all so favourable to their growth as is Musgrave's agar.

Flagellated forms, such as V. Wasielewski and Hirschfeld¹⁶ found to develop in growths of "Straw-amœbæ" transferred from agar to weak broth, were not observed in either type. In the fluid cultures no contractile vacuole was observed in the vegetative phase.

The cysts were characteristic of their respective types and similar to those found in cultures on agar.

The amœbæ above described are hardly distinguishable from many of the forms described and figured by Musgrave and Clegg and Noc; on the other hand, they differ very markedly from *Entamœba histolytica*, (Schaudinn), *E. tetragena* and *E. coli*.

They also present very distinct differences from those amœbæ found microscopically in fresh specimens of fæces.

The presence of amœbæ in a stool was diagnosed only when a body was seen throwing out pseudopodia; active amœboid movement seems to be the only absolutely safe criterion in distinguishing amœbæ from the many confusing cellular elements which may occur in fæces.

It is a notable fact that, in those cases in which motile amœbæ were, at one time or another, found microscopically in the fæces, they were not found on every occasion of examination.

Thus out of a total of 309 microscopic examinations made in 51 such cases, the presence of amœbæ was recognised on 106 occasions only. In one chronic case, out of twelve examinations during two different attacks of dysentery motile amœbæ were found only once.

In none of the five fatal cases in which amœbæ had been seen in the fæces during life, could their presence be detected in scrapings from the intestinal ulcers, made 3-18 hours after death. In the single case where, motile amœbæ not having been found during life, they were visible in scrapings from an ulcer, the autopsy had been made 3 hours after death.

In view of these facts it seems certain that, on microscopic examination, both of stools and scrapings from the mucosa taken *post-mortem*, the number of positive results recorded falls far below the actual number of amœbic infections present. Thus the proportion of amœbic infections among the cases examined in Hazaribagh is probably nearer 60 per cent. than the 19·5 per cent. quoted.

The number of amœbæ found in the stools was not observed to have any definite relation to the severity of the symptoms.

AMOEBA FOUND MICROSCOPICALLY IN THE FÆCES.

The following general description applies to those forms which were found microscopically in the fæces.

The diameter never exceeded 40 micra, and was generally 25-30 micra, though much smaller forms, down to 3-4 micra, were sometimes seen.

In the larger forms there was generally a well-marked differentiation of the plasma into a granular central endoplasm and a transparent hyaline

ectoplasm, distributed irregularly on the periphery and protruded during motion in the form of blunt pseudopodia. They were generally of a greenish grey tint.

The nucleus was often visible as a faintly-defined, rounded or oval disc about 3-6 micra in diameter, situated eccentrically in the granular endoplasm; in many cases it could not be distinguished (Fig. 28).

Circular structureless areas of a grey or faint pink tint, 1-8 micra in diameter, were often seen in the endoplasm to the number of 12 or more, the appearance being at times suggestive of partially-decolorised red cells (Fig. 29).

Bodies exactly resembling, in size and shape, normal red blood cells were often present also, sometimes packed closely together to the number of 15 or more (Fig. 30).

Many bacteria and particles of an indeterminate nature were commonly present in the endoplasm.

No definite vacuoles, either contractile or fixed, were ever observed in fresh specimens. It is interesting to remember, in this connection, that the cultivated amœbæ were found to lose their vacuoles when transferred to fluid media.

In stained specimens the nuclear chromatin is seen to be sparsely distributed in an interrupted peripheral circle with a single spot in the centre (Fig. 31).

Neither encysted nor division forms of this amœba were recognised with certainty.

Amœbæ contained in fresh specimens of faeces on microscopic slides were found to lose their motility after 1-4 hours, assuming a rounded outline with uniformly granular contents and without a definite cyst-wall; the nucleus often became temporarily more clearly visible in these spherical forms (Fig. 32).

Later it disappeared, the cell lost its granular appearance and seemed to disintegrate, being lost to view after 1-4 days.

In some six cases, faeces containing motile amœbæ were transferred to slide preparations of Musgrave's agar. They lost their motility in 1-2 hours and, although in some cases still distinguishable after five days, (Fig. 33), were never observed to form well-defined cysts or to undergo multiplication.

Conclusions.

1. That dysentery exists in this jail as a chronic recurrent disease with a period of maximum prevalence during the rainy season, and not in an acute epidemic form.

2. That in a small proportion of cases only were organisms isolated belonging to the group of the bacillus dysenteriae.

3. That, of the bacilli isolated from fourteen cases, (i) nine were mannite-fermenters and (ii) five did not ferment mannite ; within these two sub-groups there were, again, minor differences.

4. That the agglutination reaction appears to be of little value in the diagnosis of infection with organisms of the B. dysenteriae group.

5. That amœbæ can commonly be cultivated from fæces, tap water and other materials by planting them on Musgrave's medium contained in Petri plates.

6. That amœbæ of similar characters to those found in cultures from stools are, in this part of India at any rate, common inhabitants of the air, just as are moulds and other bacteria ; at least two distinct types of these amœbæ may be distinguished.

7. That these contamination amœbæ may readily gain access to (i) specimens of fæces, however carefully collected, (ii) specimens of pus or other material which has, either before or after removal from the body, been exposed to the air, and (iii) to any material after it has been planted on Musgrave's medium contained in Petri plates.

8. That the presence of amœbæ in cultures from stools on Musgrave's medium contained in Petri plates is no sound evidence of an original infection of the intestine with these organisms.

9. That in 19·5 per cent. of the cases motile amœbæ were found in the stools on microscopic examination.

10. That these intestinal amœbæ are excreted intermittently ; hence several negative results of microscopic examination do not exclude the existence of an amœbic infection.

11. That the amœbæ observed microscopically in the stools present different characters from these cultivation amœbæ, but resembling those described for the vegetative forms of *E. histolytica* (Schaudinn).

12. That this amœba does not appear to live beyond a few hours after discharge from the body, whether the fæces containing it are transferred to Musgrave's medium or not.

13. That tuberculosis of the large intestine accounts for a small proportion of dysentery cases.

14. That there remains a large proportion of dysentery cases in which at present no definite causal factor can be recognised.

15. That a mild ankylostoma infection is common, but that no serious morbid changes could be traced to it.

16. That in cases of dysentery a marked leucocytosis may occur in the absence of any evidence of complication.

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Explanation of figures.

Figures 1—19 represent cultivated amœbæ of type (a).

FIG. 1	cysts.
FIGS. 2, 3 and 13	escape of a single motile amœba from the cyst.
FIG. 4	empty cyst shell.
FIGS. 5 and 14	free motile amœbæ.
„ 6, 8 and 16	fission.
„ 9—11	formation of the “lobulate- body.”
„ 12 and 19	commencing encystment.
FIG. 17	amœbæ containing endogenous buds.
„ 18	budding forms.

Figures 20—28 represent cultivated amœbæ of type (b).

FIGS. 20 and 24	cysts.
„ 21 and 25	free motile amœbæ.
„ 22, 23 and 27	fission.
FIG. 26	group of amœbæ containing en- dogenous buds.
„ 28	vacuolated amœbæ with irre- gular chromatin masses.

Figures 29—34 represent amœbæ found microscopically in the fæces.

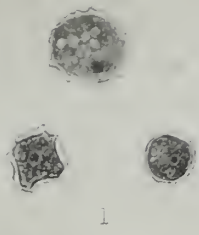
FIG. 29	motile amœbæ in a fresh speci- men of fæces.
„ 30	amœbæ showing circular area in the endoplasm.
„ 31	amœbæ showing contained bodies exactly resembling red corpuscles.
„ 32	amœbæ from fresh specimen of fæces, fixed and stained.
„ 33	amœbæ on slide 3 hours after evacuation.
„ 34	amœbæ four days after the fæces containing it had been transferred to Musgrave's agar.

- FIGS. 1—12, 20—23, 29—31, 33 and 34 . . . are unstained.
- „ 13, 15, 17—19, 25 and 32 . . . are fixed by corrosive acetic-alcohol and stained with Iron hæmatoxylin and Eosine.
- „ 14, 16, 24, 26 and 28 . . . are fixed by Osmic acid and stained by Giemsa's method.

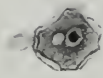
V. Wasielewski and Hirschfeld's method²⁶ of fixation was employed in dealing with cultivated amœbæ.

The drawings were made with a Zeiss's drawing apparatus, compensating ocular No. 6 and apochromatic 2 mm. aperture 130 immersion lens, tube length 160 mm.

Each division of the scale represents 2 micra.



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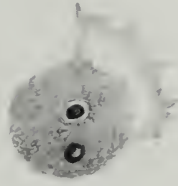
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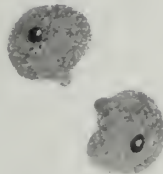
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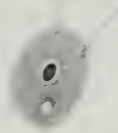
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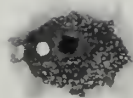
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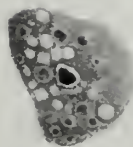
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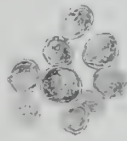


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PLATE II



19



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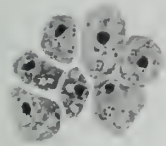
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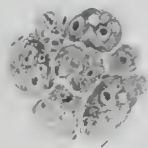
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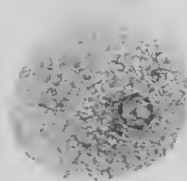
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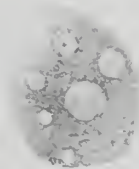
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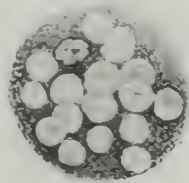


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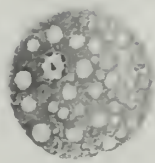


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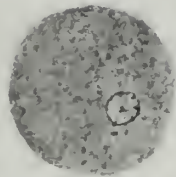
PLATE 10



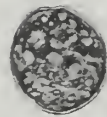
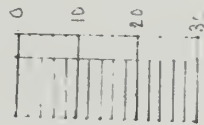
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Photomicrograph

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